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REGULATION OF METABOLIC PATHWAYS IN LIVER AND KIDNEY DURING EXPERIMENTAL DIABETES: EFFECTS OF ANTIDIABETIC COMPOUNDS

Najma Zaheer Baquer*, Dhananjay Gupta and Jayadev Raju.

Hormone and Drug Research Laboratory, School of Life Sciences, Jawaharlal Nehru University, New Delhi - 110 067.

ABSTRACT:

Diabetes has been classified as a disease of glucose overproduction by tissues, mainly liver and glucose underutilization by insulin requiring tissues like liver, adipose and muscle due to lack of insulin. There is, however, glucose over utilization in tissues not dependent on insulin for glucose transport like kidney, nerve and brain. There are serious complications due to this excess glucose in these tissues and their reversal is important for a good metabolic control and normalisation of other parameters. Insulin, trace metals and some plant extracts have been used to see the reversal effects of the complications of diabetes in liver and kidney in experimental diabetes. Almost complete reversal of the metabolic changes has been achieved in the activities of key enzymes of metabolic pathways in liver and kidney and an effective glucose control has been achieved suggesting a combination of therapies in the treatment of metabolic disturbance of the diabetic state.

KEY WORDS: Diabetes, antidiabetic compounds, glycolysis, gluconeogenesis, antioxidant enzymes.

Genesis of Diabetes Mellitus

There are two principal types of diabetes: the first is juvenile or Type I diabetes in which there is usually total or nearly total failure to secrete insulin. In the first, patients can also develop ketoacidosis. The cause of this disorder is autoimmune destruction of insulin secreting cells in the islets of Langerhans. The second major group of patients with diabetes mellitus develops a condition in which an inadequate amount of insulin is secreted even though the insulin secreting cells are intact. Type II diabetes is often familial, common form and usually develops later in life; late onset (maturity onset) develops between the age of 40 and 70 and is most common in obese subjects. Obesity has been found to lead to resistance to the insulin action.

Author for correspondence

Dr. Najma Zaheer Baquer, Professor, at above address E-mail: najma@inuniv.ernet.in

Over production of some hormones can give rise to diabetes mellitus because their actions are opposite to those of insulin. Thus in acromegaly, excessive growth hormone secretions (concentrations) may lead to diabetes. Excessive production of adrenaline, e.g. by an adrenal medulla tumour, can also give rise to diabetes as can over production of cortisol from the adrenal cortex or the administration of large amount of glucocorticoids. In the latter case, the glucocorticoids can be from the administration of pharmacological amount of glucocorticoids. Severe damage or surgery to the pancreas can also be a cause due to less production and deficiency of insulin. A disturbance of liver function may disturb carbohydrate metabolism to produce diabetes.

Virus infection may also be important, some affecting the Islets of Langerhans. Studies of human leucocytes antigen (HLA) markers have indicated that there is a relationship between the antigenic determinants and Type-I diabetes. The class II antigens, DR3 and DR4 are associated with an increased risk of developing insulin deficient diabetes.

Glucose overutilization in diabetes

The characteristic changes occurring in uncontrolled diabetes are a rise in blood glucose and increase in glycogen breakdown, gluconeogensis, fatty acid oxidation, ketone body production and urea formation. There is depression in the synthesis of glycogen, lipid and protein in the cells of those tissues that are normally dependent on insulin. Spiro in 1976 (1) pointed out that although diabetes has classically been considered a disease of glucose underutilization there are now many indications that in diabetes a shunting of glucose from insulin dependent pathways to those not requiring this hormone may take place.

Diabetes has been considered to be a disease with glucose overproduction by liver and underutilization by insulin requiring tissues such as muscle and adipose. Glucose overutilization is being recognised as an important facet of the complications of diabetes (2-5). The cells of those tissues which have an insulin dependent glucose transport system are unaffected by high blood glucose concentration in diabetic animals since the glucose transport system is not active in the absence of insulin. However, this is not so for the insulin-independent cells in which glucose entry is largely governed by the concentration gradient between the exterior and interior of the cell. In consequence, overutilization of glucose in diabetes can occur in these tissues. Thus, in diabetes there appears to be diversion of glucose from insulin dependent pathways to those not requiring the hormone. Figure 1 shows the interrelationships among alternative routes of glucose metabolism.

The facilitation of many processes in such insulin independent tissues by the raised levels of intracellular glucose may result in some of the pathological phenomenon associated with long term diabetes (Figure 2).

In uncontrolled or poorly controlled diabetes there is increased glycosylation of a number of proteins including haemoglobin and alpha-crystallin of lens. In long term diabetes the glycosylated form of haemoglobin A1c has altered affinity for oxygen and this may be a factor in tissue anoxia while the glycosylation of alpha-crystallin of lens protein may lead to cataract formation. A glucosamine protein complex is also formed in long standing diabetes resulting in biochemical and morphological alterations of the capillary system. There is some evidence that increased glycosylation of collagen is related to basement membrane thickening in the kidney (6).

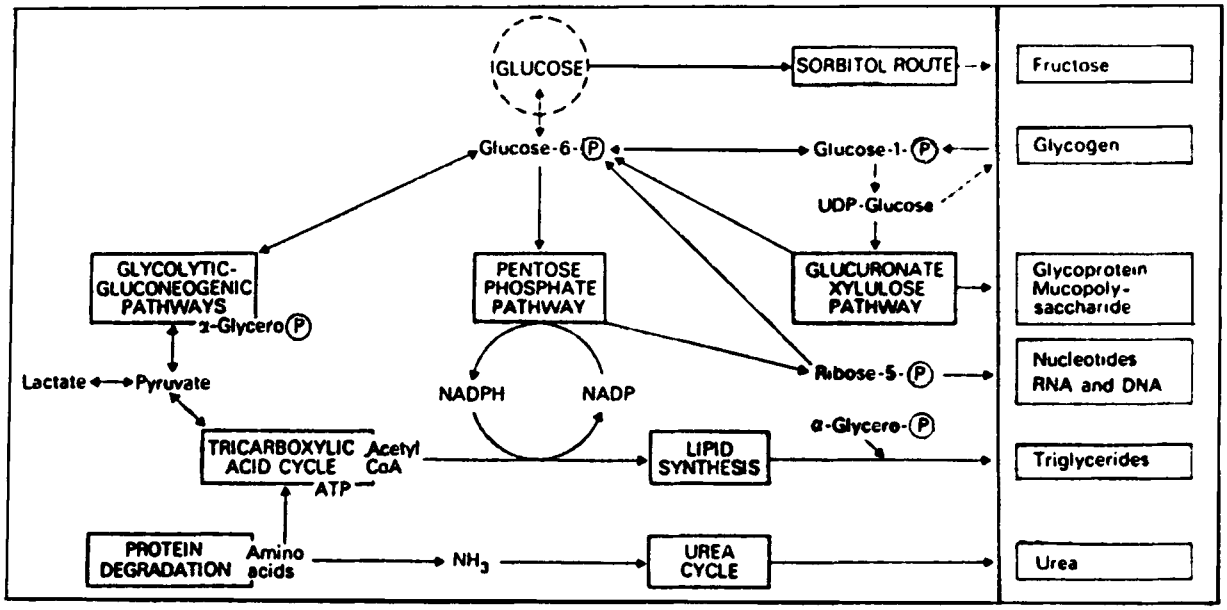

Figure 1. Interrelationships among alternative routes of glucose metabolism: The central role of glucose in carbohydrate, fat and protein metabolism.

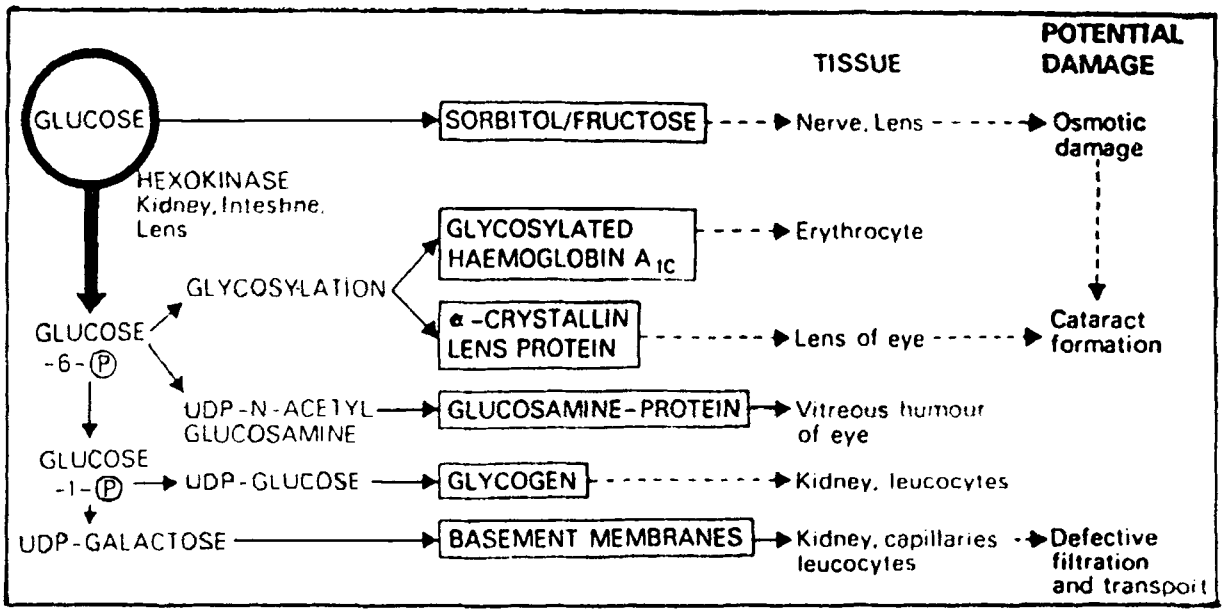

Figure 2. Glucose overutilization and induced pathological changes in tissues resulting from non-insulin requiring pathways.

Metabolic pathways affected during diabetes

A number of factors tend to promote the diversion of glucose into the sorbitol pathway leading to fructose and sorbitol accumulation (3). In diabetes the most important factors are first, the high level of intracellular glucose in tissues where the glucose transport system is insulin independent, such as lens and kidney and second, the high NADPH:NADP ratio resulting from the decrease in the rate of other reductive synthetic reactions, such as fatty acid synthesis and hydroxylation reactions. The accumulation of sorbitol could cause osmotic damage that may be important for example, in the aetiology of cataract formation. The peripheral neuropathy, and altered nerve conduction velocity, that occur in some diabetic patients, may be linked to sorbitol accumulation and associated changes in myoinositol (Figure 2).

Some of the metabolic changes include raised level of glucose-6-phosphate and derivatives of glucose-6-phosphate such as glycogen and components of basement membrane in tissues; formation of basement membrane during the growth phase; the thickening of the basement membrane in kidney and WBC occurs in diabetes; and also the increased activity of hexokinase in the kidney cortex, intestinal mucosa, lens and brain occur in diabetes (2,7,8).

In uncontrolled diabetes there is an enlargement of kidney, a rise in RNA content and in the rate of protein synthesis. There is also a change in the acid base balance and loss of electrolytes (9).

The pentose phosphate pathway (PPP) plays an essential role in providing R5-P for nucleotide and nucleic acid synthesis and NADPH for many reductive synthetic reactions. Enzymes of oxidative and non-oxidative segment of the pathways are present in kidney. The PPP may contribute both to the increased requirement of R5-P for RNA synthesis and compensatory mechanisms occurring and acid base balance (9,10).

The glucuronate-xylulose pathway is also active in kidney and produces UDP-glucose, important for the glycogen, glucuronides and mucopolysaccharides for the formation of the basement membrane. The pathway may thus be involved in implementing the increased requirement for UDPG arising from the 30-fold increase in the glycogen level in kidney and thickening of the basement membrane that occurs in uncontrolled diabetes (6,7) (Figure 3).

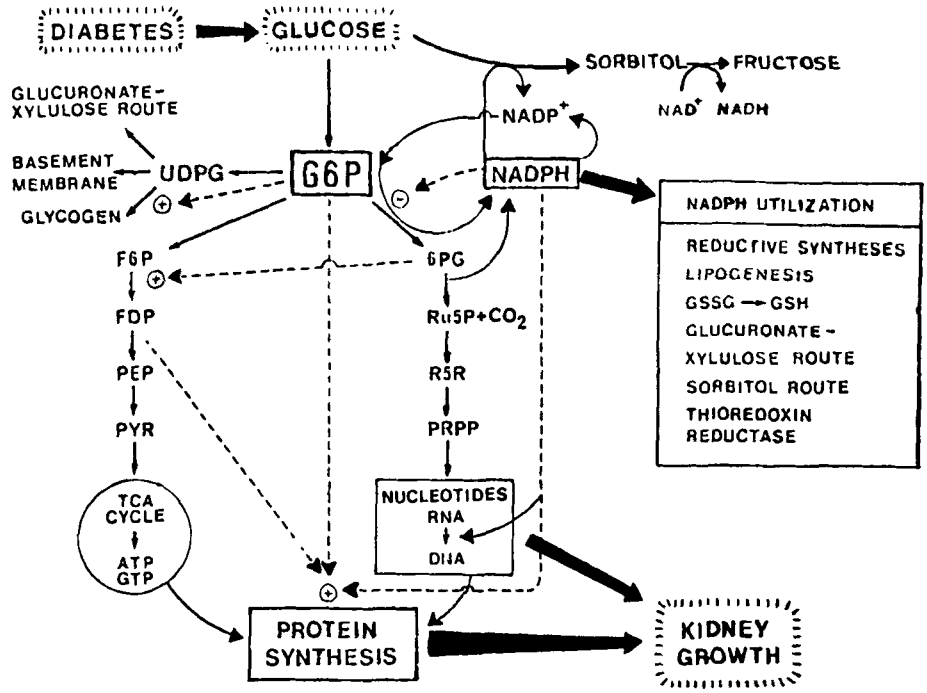

Figure 3: Biochemical network linking hyperglycaemia to kidney hypertrophy

Enzymes of free radical metabolism

Studies by Oberley (11) have shown the implication of reactive oxygen centred free radicals in the development of diabetes. Diabetic complications have also been suggested to involve free radical related processes such as the disturbed antioxidative system and oxidative damage of membrane. In diabetes, alterations in the endogenous free radical scavenging defence mechanisms may lead to ineffective scavenging of reactive oxygen species resulting in oxidative damage. The altered antioxidant status, during diabetes, causes increased production of free radicals leading to oxidative damage and tissue injury. During diabetes, liver showed a relatively more severe impairment in antioxidant capacity than kidney. They have been shown to be restored by insulin, vanadate and lithium treatments (12).

Glycolysis and other related pathways affected

A comparative survey of the enzymes of the glycolytic route, the pentose phosphate pathway, glucuronate-xylulose route and certain enzymes of the tricarboxylic acid, hydrogen shuttle systems and lipogensis in kidney and liver has been reported earlier by the authors (13).

In kidney, the majority of the enzymes of these routes kept pace with kidney growth in diabetes and remained constant when expressed as per gram kidney and showed increased activity when taken as per total kidney per 100g body weight in diabetic groups. Enzymes increasing faster than kidney weights were hexokinase (type I) and xylulose-reductase, while markedly lower activities were found for malic enzyme, malate dehydrogenase and NADP-isocitrate dehydrogenase. These increases in the kidney enzymes are in contrast with the decreased hexokinase and pentose phosphate pathway activity in the liver in diabetes (Figures 4 and 5).

A number of major differences with respect to aspects of glucose metabolism have been reported between the changes in liver and kidney in diabetes. In liver the decreased activity of hexokinase type IV (glucokinase) and enzymes of the pentose phosphate pathway have been well documented (14-16), while in kidney the activity of the dehydrogenases of the PPP have been shown to increase in diabetes (7-9). The increased activity of PPP shows a positive correlation with the rate of kidney growth in diabetes (9). The key

intermediate, glucose-6-phosphate is increased in the kidney and decreased in liver in experimental diabetes (7,17,18).

Methodology

Animals

Albino rats of Wistar strain weighing around 200 gms and approximately two months old were used. The rats were starved for 24 hours and made diabetic by subcutaneous injection of alloxan monohydrate (20mg/100gm body weight), dissolved in 0.154 M acetate buffer (pH 4.5). The treated rats were given daily dose of two IU of insulin subcutaneously for next 7 days. Insulin was then withdrawn and both groups of rats were randomly divided into experimental groups as described (19). Age matched controls were maintained. All animals were maintained for three weeks and had access to water and food *ad libitum.*

Treatment with antidiabetic compounds

Besides insulin, sodium orthovanadate, lithium carbonate, *Momordica charantia* (bitter gourd or karela) extracts and *Trigonella foenum graecum* (fenugreek or methi) seed powder were used as insulin mimetic compounds. The latter two i.e. *Momordica charantia* extracts and *Trigonella* seeds have been very widely used in India and other Asian countries for the control of blood sugar and diabetic complications and as anticholesterol agent (21-28). Murthy and co-workers (25, 26a,b, 27a,b,c) studied some aspects of mechanism of action (insulin release, key enzymes of glycolysis and gluconeogenesis, lipid profile) of orally active purified hypoglycemic compounds from these two plants. However, their detailed mechanism of action is still not fully understood. The diabetic group treated with insulin received 2 IU protamine zinc insulin intraperitoneally. Sodium orthovandate (0.6 mg/ml) was given to both the control group animals treated with vanadate and to diabetic group animals treated with vanadate in drinking water which contained 80 mM NaCl. NaCl was included to reduce the toxicity of vanadate (28). The diabetic group animals treated with *Trigonella* were fed with powdered rat feed with 5 percent powdered *Trigonella* (fenugreek) seeds. The diabetic group animals treated with both vanadate and *Trigonella* received 5 percent powdered *Trigonella* seed powder and 0.2 mg/ml sodium orthovanadate. Lithium treatment and lithium and vanadate treatment were described in our earlier paper (12).

Preparation of extracts and enzyme assays

Liver and kidney from experimental animals and age matched control animals were excised

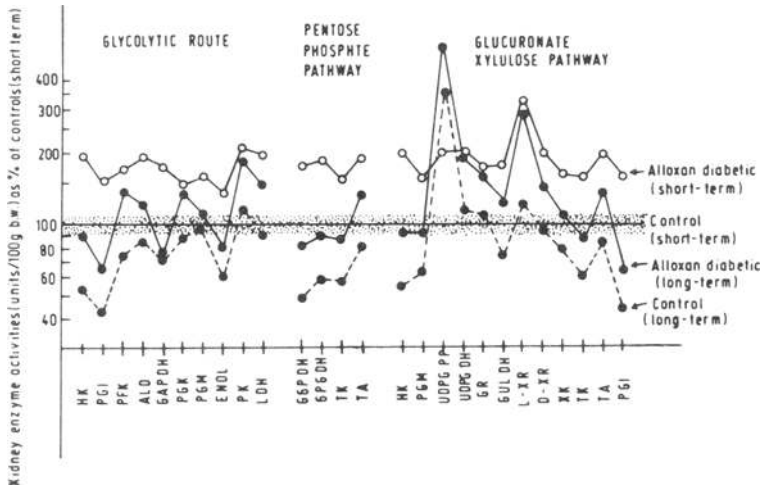

Figure 4 Profile of change in activity of enzymes of the glycolytic route, pentose pathway and glucoronate-xylulose pathway in the rat kidney in short-term and long-term (6-9 months) diabetes, based on total kidney activity 100g body weight. The profile of enzyme activity in the kidney based on the total units in the kidney 100 body weight, is shown for the short-term diabetic group (○—○), long-term diabetic group (●—●), and age matched control group for long-term diabetic rats (○—○) expressed as a percentage of the values for the short-term control group. The values are given on a log scale, the horizontal line and shaded area indicate the short-term control group. Abbreviations used are as follows: HK, hexokinase; PGI, phosphoglucose isomerase; PFK, phosphofructokinase; ALD, aldolase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; PGK, phosphoglycerate kinase; PGM, phosphoglycerate mutase; ENOL, enolase; PK, pyruvate kinase; LDH, lactate dehydrogenase; G6PDH, glucose-6-phosphate dehydrogenase; 6PGDH, 6-phosphogluconate dehydrogenase; TK, transketolase; TA, transaldolase; GR, glucuronate reductase; GULDH, gulonate dehydrogenase; L-XR, L-xylulose reductase; D-XR, D-xylulose reductase; XK, xylulosekinase

rapidly following decapitation. Homogenates were prepared in 0.25 M sucrose, 0.02 M TEA buffer pH 7.4 containing 0.12 mM dithiothreitol (DTT) using a Potter Elvehjem homogenizer fitted with a teflon plunger. Other details were as described in earlier papers for determination of enzyme and metabolite levels (7,8,9,13).

Assay of gluconeogenic and related enzymes

The activities of the gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase were measured as described in our earlier paper (29) by measuring the amounts of phosphate formed using glucose-6-phosphate and fructose-1, 6-bisphosphate as substrates. For assay of both the enzymes the soluble fractions of tissue homogenates were used. All the procedures for the preparation of extracts are as described earlier (13). Glyoxalase I activity in liver and kidney cytosolic fractions was assayed as described by Racker (30).

Vanadium and insulin determination

For vanadium estimation the procedures used were as described earlier (19). The concentration of vanadium was analysed by inductively coupled plasma atomic emission spectrometer (Perkin Elmer Plasma 40 Model). The plasma insulin levels were quantitated by radioimmmunoassay using a radioimmunossay kit supplied by Bhabha Atomic Research Center (BARC), Mumbai India.

Calculation of enzyme units

Enzyme units for glucose-1,6-bisphosphatase and fructose-1,6-bisphosphatase were calculated and defined as reported earlier (29). The glyoxalase I enzyme units were calculated and defined as described by Racker (30).

Glucose utilization and antidiabetic effects of vanadate, Trigonella and Momordica

General parameters of all the groups of rats are summarized in Tables 1a and 1b. Alloxan induced experimental diabetes caused a four fold-increase in blood glucose levels which was restored to normal values after 3-4 days of vanadate treatment, this was continued till the animals were used for the experiments.

Insulin-like effects of vanadate

In intact cells, vanadium compounds possess insulin-like effects. They mimic both glucose

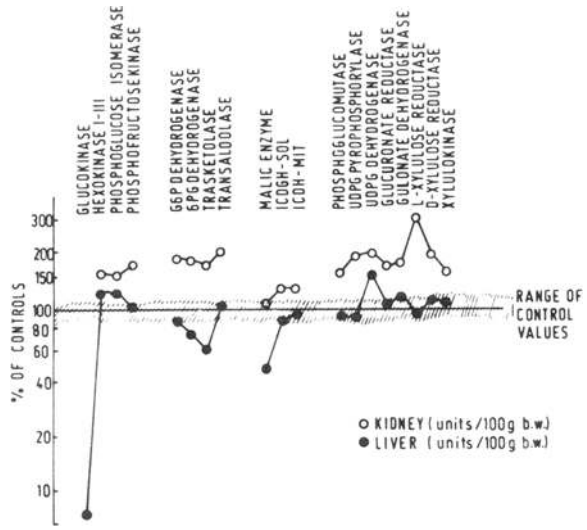

Figure 5. Effect of alloxan diabetes on enzymes of the pentose phosphate pathway and glucuronate-xylulose pathway in kidney and liver. To facilitate comparision, the results of the diabetic group are expressed as a percentage of the control values and these results are shown on a logarithmic scale. The basis for the values shown is total enzymes units in the organ per 100 g body weight. The shaded area across the base line indicates the average SI for the control group. (●) Liver from alloxan-diabetic rats. (○) Kidney from alloxan-diabetic rats (short-term diabetes). ICDH-SOL and ICDH-MIT represent cytosolic and mitochondrial isocitric dehydrogenases, respectively.

Table 1a . General parameters: plasma and kidney vanadium and plasma insulin levels of different experimental groups of rats

	Control	Control + Vanadate	Diabetic	Diabetic + Vanadate
General parameters				
Body Weight (g)	265 ± 5	244 ± 6[a]	193 ± 6a	200 ± 4[a]
Kidney weight (g)	1.74 ± 0.03	169 ± 0.03[a]	2.29 ± 0.04	170 ± 0.04[a]
Kidney Weight/100g Body Weight	0.66 ± 0.02	0.69 ± 0.02[a]	1.20 ± 0.04[a]	0.86 ± 0.01[a]
Blood glucose (mg/dl)	105 ± 4	92 ± 3[a]	436 ± 11[a]	109 ± 5
Fluid Intake (ml/dl)	36 ± 3	30 ± 3[b]	210 ± 10[a]	32 ± 2c
Vanadium intake (mg/Kg/day)	0	74 ± 5	0	92 ± 8
Vanadium Level				
Plasma	<0.2	1.65 ± 0.5	<0.2	188 ± 0.7
Kidney	<0.2	6.65 ± 1.25	<0.2	7.20 ± 1.32
Plasma Insulin (uU/ml)	15.90 ± 1.65	8.90 ± 0.90[a]	4.11 ± 0.61[a]	5.00 ± 0.81[a]

Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a]p <0.001, [b]p <0.01, [c]p <0.025 and [d]p <0.05. Details of treatments with antidiabetic compounds and definition of units are described in Methodology.

Vanadium intake was measured as 1000 x [average fluid intake per day (ml) x Concentration of vanadium (mg/ml) body weight (g)].

Table 1b : General parameters: changes in body weight, tissue weight, blood glucose and tissue protein content of different experimental rat groups.

	Control	Diabetic	Diabetic + Insulin	Diabetic + Vanadate	Diabetic + Trigonella	Diabetic + Vanadate + Trigonella
Body Weight (g)	216 ± 9.0	145 ± 7.6[a]	186 ±2.3[b]	183 ±1.5[b]	200 ± 2.9	200 ± 3.3[d]
Liver Weight (g)	5.5 ± 0.1	4.9 ± 0.1[d]	5.4 ± 0.1	5.5 ± 0.0	5.8 ± 0.1	5.8 ± 0.1[d]
Liver Weight (g)/100g Body Weight	2.55	3.37	2.90	3.00	2.90	2.86
Kidney Weight (g)	1.1 ± 0.04	1.5 ±0.04[b]	1.38 ± 0.04	1.2 ± 0.4	1.4 ±0.07	1.3 ± 0.05
Kidney Weight (g)/100g Body Weight	0.51	1.03	0.74	0.66	0.70	0.64
Blood Glucose (mg/dl)	102.6 ± 5.0	317.6 ± 16[a]	111.6 ± 0.4	104 ± 0.8	106.0 ± 3.3	105.0 ± 4.1
Protein Soluble (mg/ml)						
Liver	121 ± 0.1	156 ± 0.2[b]	103 ± 0.1	117 ± 05[b]	130 ±0.2[b]	124 ± 0.5[b]
Kidney	92.2 ± 0.1	88.7 ± 0.1[c]	91.5 ± 0.1	85.1 ± 0.3[a]	86.2 ±0.4[c]	88.9 ± 0.1[c]

Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a]p <0.005, [b]p <0.05, [c]p <0..1 and [d]p <0.5. Details of treatments with antidiabetic compounds are given in Methodology.

Table 2. Insulin-like actions mediated by sodium orthovanadate in insulin responsive tissues.

Activity	Direction of Effect	Target Tissue
Hexose transport	Stimulated	Rat adipocytes, skeletal muscle, brain, liver.
Glucose oxidation	Stimulated	Rat diaphragm, adipocytes, liver
Gluconeogenesis	Inhibited	Rat liver.
Glycogenesis	Stimulated	Rat hepatocytes, skeletal muscle, adipocytes.
Glycogenolysis	Inhibited	Rat liver, skeletal muscle.
Lipogenesis	Stimulated	Rat adipocytes.
Lipolysis	Inhibited	Rat adipocytes.
Ketogenesis	Inhibited	Rat liver.
Urea cycle enzymes	Inhibited	Rat liver.
Mitogenic activity	Augmented	Various cultured cells
Translocation of IGF-II	Stimulated or Augmented	Rat adipocytes
K^+uptake	Stimulated	Cardiac muscle cells.
$Ca^{2+}Mg^{2+}$ ATPase	Inhibited	Plasma membranes from rat adipocytes.
Ca^{2+}influx	Stimulated	Adipose tissue.
Intracellular pH	Elevated	A-431 cells.
ATPase	Inhibited	Rat liver.
Cytoplasmic protein		
Tyrosine kinase	Stimulated	Rat adipocytes.

Modified from Shechter, 1995 (31).

transport and the intracellular action of insulin in skeletal muscle. They enhance glycogen synthesis, inhibit ACTH induced lipolysis in adipocytes, interact with growth factors such as EGF, insulin and insulin-like growth factors II (Table 2). These actions of vanadate have been discussed in detail by Shechter et. al. (31) and Sekar et. al. (32). Kidney, a glucose overutilizing tissue and liver a glucose underutilizing tissue, were studied for the reversal effects of experimental diabetes by vanadate, insulin and other insulin mimetic compounds.

Polyol pathway enzymes in the kidney

The polyol pathway is a minor pathway of glucose metabolism and under normal conditions there is little flux through the pathway in most tissues. However, in diabetic hyperglycemic conditions the flux through this pathway increases dramatically leading to increased intracellular concentrations of sorbitol in tissues like kidney, erythrocyte and lens. Because of the impermeability of most cells to sorbitol, the accumulation of this metabolite establishes an osmotic imbalance that ultimately causes the development of a number of pathological conditions in various tissues (33-35). The changes in the activity of polyol pathway enzymes sorbitol dehydrogenase (SDH), aldose reductase (AR) in kidney cortex and medulla of the diabetic and vanadate treated rats are given in Table 3, together with sorbitol and glucose levels. The AR activity was found to be about two folds higher in medulla than in the cortex, SDH was very low in medulla and high in the cortex. A 35 percent increase in SDH in cortex was found in diabetes with no change in the medulla. Sorbitol formation was much higher in medulla where it accumulated 30 fold higher than cortex. Vanadate administration to the diabetic rats significantly lowered the elevated sorbitol content in renal medullary region and also caused a marked decline in glucose levels which was elevated by 9 and 6.7 folds in cortex and medulla during diabetes (19). The percentage changes in the activities of aldose reductase and sorbitol dehydrogenase and

Table 3. Changes in enzyme activities and metabolite levels of polyol pathway in rat renal medullary and cortical regions of different experimental groups: Effect of vanadate

	Control	Control + Vanadate	Diabetic	Diabetic + Vanadate
Enzyme Activities				
Sorbitol dehydrogenase				
Cortex	6.91 ± 0.12	6.34 ± 0.14[a]	9.35 ± 0.15[a]	6.71 ± 0.12[a]
Medulla	0.12 ± 0.01	0.12 ± 0.01	0.13 ± 0.01	0.12 ± 0.01
Aldolase reductase				
Cortex	0.20 ± 0.01	0.19 ± 0.02	0.26 ± 0.01[a]	0.20 ± 0.01
Medulla	0.44 ± 0.01	0.42 ± 0.01[b]	0.62 ± 0.01[a]	0.48 ± 0.01
Metabolites				
Sorbitol				
Cortex	0.22 ± 0.02	0.21 ± 0.01	0.26 ± 0.02[c]	0.23 ± 0.02
Medulla	6.40 ± 0.54	6.28 ± 0.41	11.50 ± 0.63[a]	7.36 ± 0.45[c]
Glucose				
Cortex	2.56 ± 0.13	2.46 ± 1.17	22.26 ± 1.37[a]	3.04 ± 0.28
Medulla	0.32 ± 0.02	0.29 ± 0.03[d]	21.30 ± 1.04[a]	0.40 ± 0.04

Enzyme activity is expressed as units per gram tissue weight per minute. Metabolite level is expressed as µmol per gram tissue weight. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a]p<0.001, [b]p<0.005, [c]p<0.01 and [d]p<0.025. Details of treatments with antidiabetic compounds and definition of units are descried in Methodology.

metabolite levels in the whole kidney during diabetes and its reversal by insulin and vanadate are given in Figures 6 and 7.

Glycolytic enzymes in liver and kidney

A comparative study was made to elucidate the in vivo antidiabetic effects of vanadate on glycolytic enzymes, hexokinase isoenzymes, pyruvate kinase and lipogenic enzyme, malic enzyme in both kidney and liver. The results of these experiments are shown in Table 4. In liver, a tissue with glucose underutilization, hexokinase isoenzyme II and glucokinase were almost completely depleted during diabetes together with pyruvate kinase (PK) and malic enzyme, the other insulin dependent enzyme, as reported earlier (36). The administration of vanadium completely restored the enzyme levels to almost normal values. In kidney, which overutilizes glucose in consistent hyperglycaemia during diabetes, the enzymes were found to increase except for malic enzyme.

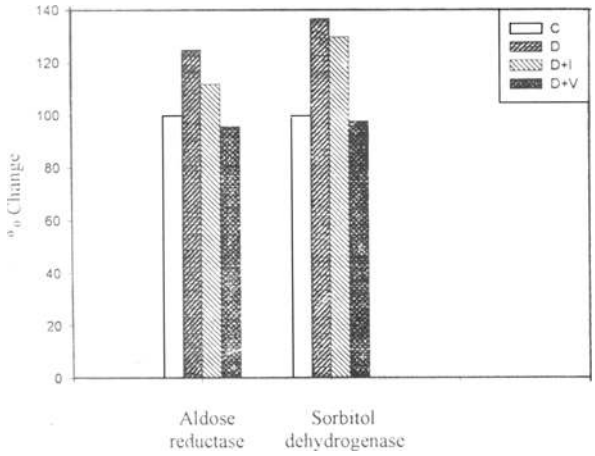

Figure 6. Percent change in the activities of aldose reductase and sorbitol dehydrogenase in whole kidney of control (C), diabetic (D), diabetic treated with insulin (D-I) and diabetic treated with vanadate (D-V) rats

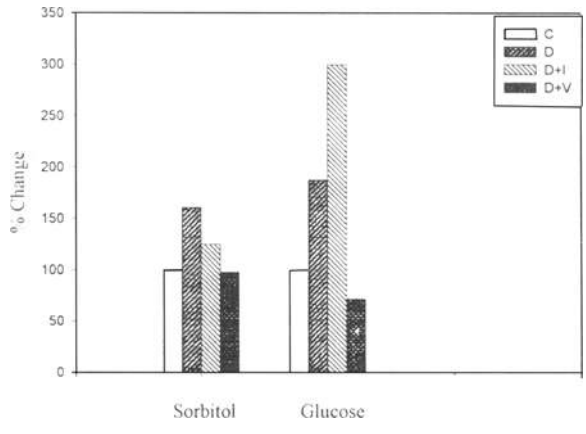

Figure 7: percent change in the levels of sorbitol and glucose in whole kidney of control (C), diabetic (D), diabetic treated with insulin (D+I) and diabetic treated with vanadate (D+V) rats

Table 4. Change in the activities of hexokinase isozymes, pyruvate kinase and malic enzyme in rat liver and kidney of different experimental groups: Effect of vanadate.

	Control	Control + Vanadate	Diabetic	Diabetic/ Control (%)	Diabetic + Vanadate	Diabetic + Vanadate /Control (%)
Kidney						
Hexokinase						
Cytosolic						
Total	0.64 ± 0.04	0.59 ± 0.03	0.88 ±0.06[a]	138	0.68 ± 0.03	106
Type I	0.41 ± 0.02	0.40 ± 0.03	0.58 ±0.05[a]	141	0.44 ± 0.02	107
Type II	0.23 ± 0.02	0.23 ± 0.02	0.30 ±0.02	130	0.24 ± 0.01	104
Mitochondrial						
Total	0.47 ± 0.04	0.40 ±0.01[c]	0.58 ±0.04[b]	123	0.49 ± 0.03	104
Type I	0.24 ± 0.01	0.24 ± 0.02	0.30 ±0.02	125	0.27 ± 0.01	113
Type II	0.23 ± 0.03	0.21 ± 0.01	0.29 ±0.04	126	0.22 ± 0.03	96
Pyruvate kinase	8.13 ± 0.75	7.57 ± 0.87	13.1 ±1.57[b]	161	9.14 ± 0.6	112
Malic enzyme	1.08 ± 0.13	1.01 ± 0.09	1.15 ±0.09	106	1.17 ± 0.08	108
Liver						
Hexokinase						
Cytosolic						
Total	9.47 ± 0.47	9.18 ± 0.62	2.81 ±0.12[a]	29	9.09 ± 0.62	97
Type I	1.44 ± 0.10	1.47 ± 0.15	0.96 ±0.07[a]	67	1.39 ± 0.14	97
Type II	1.32 ± 0.04	1.12 ±0.05[a]	0.46 ±0.04[a]	35	1.26 ± 0.11	95
Type IV	6.98 ± 0.34	6.58 ± 0.47	1.39 ±0.15[a]	20	6.81 ± 0.58	98
Pyruvate kinase	106 ± 7	94 ± 5	68 ± 6[a]	64	94 ± 6[d]	89
Malic Enzyme	4.50 ± 0.43	4.19 ± 0.31	2.32 ±0.21[a]	56	4.24 ± 0.21	94

Enzyme activity is expressed as total units per 100g body weight in all cases. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a] p <0.001, [b] p <0.005, [c] p <0.01 and [d] p <0.025. Details of treatments with antidiabetic compounds are described in Methodology.

Vanadate administration restored the elevated enzyme activities in kidney of diabetic rats to almost normal values. Similar changes on the effect of diabetes have been reported in our earlier papers (7,13).

Free radical scavenging enzymes

In view of the various insulin mimetic effects of vanadate, the present experiments were designed to show and study the efficacy of vanadate treatment in controlling the altered antioxidant status of the liver and kidney of diabetic rats. The results of the changes of enzymes glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), superoxide dismutase (Cu-Zn-SOD and Mn-SOD) activities, reduced glutathione (GSH) and oxidised glutathione (GSSG) levels in liver of different experimental groups are shown in Tables 5 and 6.

A generalised decrease in the above-mentioned enzymes was found, as has been reported earlier (37). Oral administration of vanadate to diabetic rats almost completely normalised the 30% depressed GPx and Mn-SOD levels in liver. The Cu-Zn-SOD was however only partially restored. The CAT levels and GSH levels were not normalised. GR activity did not change much during diabetes as was GSSG level, both were unaffected by the trace metal treatments.

The altered tissue anti-oxidant status due to diabetes may cause increased production of free radicals leading to oxidative damage and tissue injury (11). Liver, during diabetes, showed a relatively more severe impairment in antioxidant capacity than the kidney. The data indicate clearly that trace element therapy, either alone or in combination may prove effective in the treatment of metabolic disturbances of the diabetic state. However, the mechanism of action remains unclear. It has been suggested that the effects of vanadate are due to oxo-vanadium compounds which

Table 5. Changes in glutathione peroxidase (GPx), glutathione reductase (GR), catalase (CAT), Cu-Zn-superoxide dismutase (Cu-Zn-SOD) and Mn-superoxide dismutase (Mn-SOD) activities; and reduced glutathione (GSH) and oxidised glutahione (GSSG) levels in rat liver of different experimental groups: Effect of vanadate.

	Control	Control + Vanadate	Diabetic	Diabetic + Vanadate
Enzyme Activities				
GPx	22.1 ± 1.6	20.1 ± 1.8	15.4 ± 1.5[c]	21.8 ± 1.9
GR	6.8 ± 0.2	6.0 ± 0.2[c]	6.6 ± 0.2	6.4 ± 0.3
CAT*	34.0 ± 2.2	27.4 ± 2.8	24.0 ± 1.7[b]	20.9 ± 2.1[b]
Cu-Zn-SOD	2238 ± 276	2077 ± 270	1333 ±174[c]	1682 ± 184
Mn-SOD	225 ± 22	213 ± 17	162 ± 18[c]	220 ± 17
Metabolite Levels				
GSH	5.5 ± 0.4	4.2 ± 0.5[c]	3.8 ± 0.4[c]	3.0 ± 0.3[a]
GSSG	0.18 ±0.03	0.16 ± 0.02	0.19 ±0.03	0.17 ±0.03

Enzyme activity is expressed as units per gram tissue weight per minute. Metabolite level is expressed as µmol per gram tissue weight. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a] $p < 0.001$, [b] $p < 0.01$ and [c] $p < 0.05$. Details of treatments with antidiabetic compounds and definition of units are described in Methodology.

* Activity of CAT is expressed in U/gx10^3

Table 6. Changes in the activities of catalase (CAT), glutathione peroxidase (GPx), glugathione reductase (GR), superoxide dismutase (SOD) and reduced glutathione (GSH) levels in rat liver and kidney of different experimental groups: Effect of lithium and vanadate

	Control	Diabetic	Diabetic + Lithium	Diabetic + Lithium + Vanadate
Liver				
CAT*	34.2 ± 2.4	24.2 ± 2.4[b]	33.1 ± 2.4	34.8 ± 2.1
GPx	22.8 ± 2.1	16.5 ± 1.5[b]	21.6 ± 1.9	23.8 ± 1.8
GR	6.8 ± 0.3	6.6 ± 0.2	6.3 ± 0.2	6.5 ± 0.4
Cu-Zn-SOD	2173 ± 208	1365 ±132[a]	1381 ± 140[b]	1838 ± 179
Mn-SOD	225 ± 22	172 ± 17	166 ± 15	199 ± 20
GSH	5.2 ± 0.4	3.6 ± 0.4[b]	3.9 ± 0.3[b]	3.4 ± 0.4[b]
Kidney				
CAT*	12.8 ± 1.2	7.5 ± 1.4[b]	7.7 ± 1.2[b]	9.2 ± 1.1
GPx	6.1 ± 0.6	10.1 ± 0.9[a]	9.8 ± 0.5[a]	8.7 ± 0.5[a]
GR	5.3 ± 0.4	5.9 ± 0.3	5.9 ± 0.2	5.7 ± 0.4
Cu-Zn-SOD	1131 ± 135	829 ± 79	811 ± 77	930 ± 81
Mn-SOD	125 ± 12	137 ± 19	135 ± 16	132 ± 14
GSH	2.1 ± 0.3	2.3 ± 0.3	2.2 ± 0.2	2.3 ± 0.3

Enzyme activity is expressed as units per gram tisue weight per minute. Metabolite level is expressed as µmol per gram tissue weight. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a] $p < 0.01$ and [b] $p < 0.05$. Details of treatments with antidiabetic compounds and definition of units are described in Methodology.

* Activity of CAT is expressed in U/gx10^3.

involve a superoxide anion (38,39). Vanadate mimics the action of insulin in *vivo* and in *vitro* via alternative biochemical pathways, mainly by inducing normoglycemia, improving glucose homeostasis and sensitizing target tissues to insulin. It was concluded that vanadate is capable of elevating the levels of key enzymes of fat and glucose metabolism and that it may do so at low concentrations, in this respect it may be superior to insulin (40,41). The data from our laboratory shows that besides the insulin requiring tissues, vanadate also modulates the enzymes in the insulin independent tissue like kidney thereby causing an overall normalization of the changed metabolic state of the animal. The results discussed are from our earlier work (12,37). Mechanism distal to the insulin receptor may also play a role (42).

Urea cycle enzymes and transaminases

The changes in the activities of urea cycle enzymes and transaminases during diabetes have been shown earlier (13,43). The effect of vanadate and insulin treatment on the activities of alanine aminotransferases (AlaAT), aspartate amino transferases (AspAT), arginase and glutamate dehydrogenase from rat kidney are shown in Tables 7,8.

The enzymes of gluconeogensis and protein catabolism increase in diabetes leading to cellular increases in some amino acids and TCA cycle intermediates. The aminoacids are deaminated and the carbon is used as an energy source. Ammonia is excreted by the urea cycle. The AlaAT activity increased in diabetic kidney and the administration of insulin or vanadate was found to restore normal activity in the soluble fractions only. The activity of AspAT increased in cytosolic and mitochondrial fractions. Both vanadate and insulin again restored only the cytosolic enzymes to normal values. The activities of these two transaminases may be regulated by the normalization of the redox-state i.e. NADH/NAD of the tissue by vanadium administration and also the availability of substrates for enzymes. It has been shown earlier that insulin administration to diabetic animals reverses the reduced redox state to

Table 7. Changes in the activities of alanine aminotransferases (AlaAT), aspartate aminotransferases (AspAT), arginase and glutamate dehydrogenase (GLDH) enzyme activities in short and long term experimental diabetic kidney: Effect of vanadate.

		Control	Diabetic	Diabetic + Insulin	Diabetic + Vanadate
Short term diabetes					
AlaAT	S	0.93 ± 0.17	1.62 ± 0.16[b]	0.99 ± 0.18[a]	0.87 ±0.14[a]
	M	0.69 ± 0.14	1.60 ± 0.18[b]	1.02 ± 0.15	0.99 ±0.11[a]
AspAT	S	19.35 ± 2.6	31.16 ± 3.9[b]	21.80 ± 2.6[b]	19.90 ± 2.2[c]
	M	13.20 ± 1.1	27.60 ± 2.8[a]	21.50 ± 2.3[b]	22.80 ± 2.4[b]
Arginase	S	32.60 ± 3.4[b]	46.10 ± 4.6[a]	45.90 ± 4.9[a]	34.40 ± 3.4[a]
	M	43.40 ± 4.6	78.70 ± 11.8[a]	73.30 ± 10.4	64.70 ± 4.6[a]
GLDH	M	8.80 ± 1.6	17.70 ± 2.2[b]	10.90 ± 1.4[a]	9.50 ± 1.3[b]
Long term diabetes					
AlaAT	S	1.18 ± 0.16	1.98 ± 0.10[b]	1.23 ± 0.18[a]	1.02 ±0.16[a]
	M	1.02 ± 0.18	1.83 ± 0.14[a]	1.55 ± 0.16[a]	1.59 ±0.18[b]
AspAT	S	19.90 ± 2.6	30.80 ± 3.7[c]	21.90 ± 2.5[a]	19.30 ± 2.2[b]
	M	9.30 ± 1.6	19.60 ± 1.9[a]	21.40 ± 2.1[a]	21.30 ± 2.4[b]
Arginase	S	34.70 ± 4.6	44.70 ± 5.4[b]	42.30 ± 4.2	34.40 ± 3.6[a]
	M	47.80 ± 5.8	75.90 ± 12.2[b]	76.40 ± 11.8[a]	62.90 ± 8.2[a]
GLDH	M	14.50 ± 1.8	32.70 ± 3.4[a]	20.50 ± 2.6[b]	33.40 ± 3.8[a]

Enzyme activity is expressed as units per gram tissue weight per minute. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a] $p < 0.001$, [b] $p < 0.005$ and [c] $p < 0.01$. Details of treatments with antidiabetic compounds and definition of units are described in Methodology.
S = Soluble fraction and M = Mitochondrial fraction.

control values (17). Similarly, the increased activity of mitochondrial glutamate dehydrogenase reverses to control levels with insulin and vanadate (44-46).

Arginase activity in kidney is present in both soluble and mitochondrial fractions resulting in the generation of increased levels of ammonia, pyruvate and lactate. The administration of vanadate reversed the increased arginase activity only in soluble fraction of the kidney. Vanadate and insulin treatment were found to restore the activities of AlaAT and AspAT in soluble fraction of the diabetic kidney, whereas mitochondrial AlaAT and AspAT were unaffected by either of these treatments.

Slot blot assay was performed to estimate the levels of mRNA transcripts specific to arginase in the liver under the experimental conditions of diabetes and diabetes treated with insulin and vanadate. Arginase cDNA was used as a probe to detect the arginase mRNA. Results showed that the mRNA concentrations in the liver of diabetic animals were much higher as compared to control animals, and diabetic animals treated with insulin and vanadate. The increased activity of arginase in diabetes can be due to increase in arginase specific transcript to arginase and that the arginase gene is modulated at the transcriptional level in liver (46,47).

In vitro effects of *Momordica charantia* extracts on the activities of urea cycle enzymes, arginase and the transaminases are given in Tables 8 and 9 and in Figures 8 and 9 respectively. The increased activities of these enzymes in diabetes are reversed to almost control levels with vanadium and lithium treatment as well as with *Momordica charantia* extracts in liver (Figures 8 and 9). The enzymes in the kidney however did not show a reversal to control values (Table 8). All the four enzymes showed increase in activities with ageing in diabetes as compared with age matched controls. As discussed and emphasised in our earlier papers diabetes may be said to be a hastened process of ageing.

Gluconeogenic enzyme changes in liver and kidney

Two gluconeogenic enzymes, glucose-6-phosphatase (G-6-Pase) and fructose-1,6-phosphatase (F-1,6-bisPase) have been measured in the livers and kidneys of diabetic animals and those treated with vanadate and *Trigonella* seed powder separately and in combination (Table 9). Both enzymes showed an increase in activity during diabetes in liver and kidney as has been reported earlier by Shibib *et. al.* (21). Vanadate administration was found to reverse both enzymes

Table 8. Changes in the activities of alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT), arginase and glutamate dehydrogenase activities during experimental diabetes: Effects of lithium with vanadate and *Momordica charantia* extract

		Control	Diabetic	Diabetic + Vanadate+Lithium	Diabetic + *Momordica*
AlaAT	S	0.93 ± 0.1	1.7 ±0.17^c	1.08 ±0.16^a	0.99 ±0.18^a
	M	1.64 ±0.16	0.71 ± 0.1^b	1.12 ±0.18^b	1.08 ±0.11^a
AspAT	S	20.2 ± 3.1	31.3 ± 4.0^b	19.9 ± 2.1^c	21.1 ± 2.9^a
	M	13.6 ± 1.2	28.2 ± 2.9^c	19.6 ± 2.6^a	21.8 ± 2.2^a
Arginase	S	34.4 ± 3.1	46.6 ± 5.2^c	36.1 ± 4.0^c	37.3 ± 3.1^b
	M	45.0 ± 4.3	78 ± 6.3^c	65.9 ± 4.4^b	63.1 ± 5.0^a
GLDH	M	8.9 ± 1.3	18.2 ± 1.8^c	9.5 ± 1.4^a	10.9 ± 1.5^b

Enzyme activity is expressed as units per gram kidney weight per minute. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are ^{a} p<0.001, ^{b}p<0.05 and ^{c}p<0.01. Details of treatments with antidiabetic compounds and definition of units are described in Methodology. S = Soluble fraction and M = Mitochondrial fraction.

Table 9. Changes in the activities of glucose-6-phosphatase (G-6-Pase), Fructose-1, 6-bisphosphatase (F-1,6-bisPase) and glyoxalase I in liver and kidney cytosolic fractions of experimental rats: Effects of insulin, vanadate and *Trigonella* seed powder.

	Control	Diabetic	Diabetic + Insulin	Diabetic + Vanadate	Diabetic + Trigonella	Diabetic + Vanadate + Trigonella
G-6-Pase						
Liver	11.0 ± 0.3	29.2 ± 1.6[a]	13.2 ± 0.9[b]	13.9 ± 2.1[b]	14.3 ± 0.8[b]	11.8 ± 0.6
Kidney	12.5 ± 0.8	18.3 ± 0.6[a]	12.2 ± 0.3	15.4 ± 1.0[c]	13.2 ± 0.6	11.2 ± 0.3[d]
F-1, 6-bisPase						
Liver	3.8 ± 0.2	11.6 ± 2.1[b]	5.7 ± 0.2[b]	6.3 ± 0.3[b]	6.7 ± 0.4[a]	6.2 ± 0.3
Kidney	4.1 ± 0.1	10.9 ± 0.6[a]	5.6 ± 0.0[c]	5.7 ± 0.2[a]	6.6 ± 0.4[a]	6.7 ± 0.0[a]
Glyoxalase I						
Liver	48.9 ± 3.9	29.0 ± 0.9[b]	46.1 ± 2.6	41.5 ± 3.0	41.3 ± 1.5	47.5 ± 4.0
Kidney	30.9 ± 1.2	47.4 ± 1.1[b]	32.3 ± 1.9	31.5 ± 2.6	31.2 ± 2.1	31.5 ± 1.7

Enzyme activity is expressed as units per gram tissue per minute. Each value is a mean ± SEM of four or more separate determinations. Fisher's p values are [a]p<0.001, [b]p<0.05 and [c]p<0.01. Details of treatments with antidiabetic compounds and definition of units are described in Methodology.

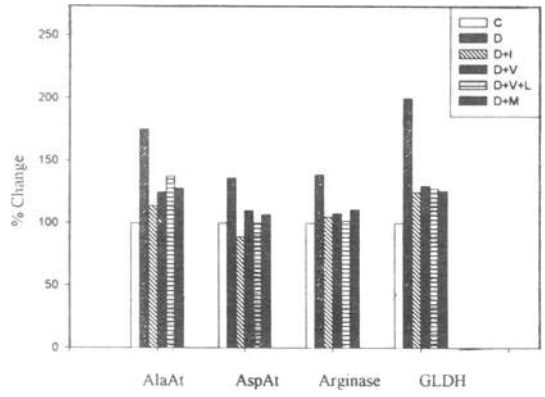

Figure 8: Percent change in the activities of mitochondrial alanine aminotransferase (AlaAT), aspartate amino transferase (AspAT), arginase and glutamate dehydrogenase (GLDH) in liver of control (C), diabetic (D), diabetic treated with insulin (D+I), diabetic treated with vanadate (D+V), diabetic treated with vanadate and lithium (D+V+L) and diabetic treated with *Momordica charantia* rats.

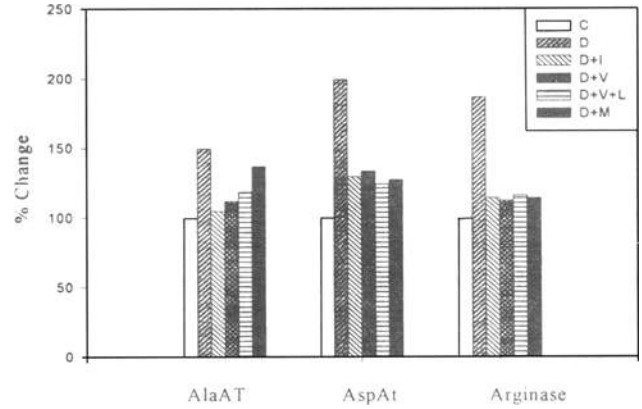

Figure 9: Percent change in the activities of cytoplasmic alanine aminotransferase (AlaAT), aspartate aminotransferase (AspAT) and arginase in liver of control (C), diabetic (D), diabetic treated with insulin (D+I), diabetic treated with vanadate (D+V), diabetic treated with vanadate and lithium (D+V+L) and diabetic treated with *Momordica charantia* (D+M) rats.

to almost control levels. Since vanadate administration is known to cause toxicity in animals, the administration of other natural ingredients, *Trigonella* seed powder was carried out. *Trigonella* seed powder, when administered alone and in combination with vanadate, was found to be the most effective in reversing both enzymes to normal values in liver and kidney. It was observed that the *Trigonella* seed powder treatment minimised the toxicity of vanadium and increased the survival rate and body weight of the animals.

The increase in activity of both the gluconeogenic enzymes in liver was found to be nearly two times higher than in kidney. The activity of G-6-Pase was found to reverse almost completely in comparison to F-1,6-bisPase in both tissues. This may be due to the fact that F-1,6-bisPase has multiple regulators than G-6-Pase. The increase in F-1,6-bisPase in liver may be due to the changes

in the allosteric effectors of the enzymes namely fructose-2,6-bisphosphate, ATP, AMP and citrate. In a diabetic state there is more lipolysis than lipogenesis, especially in liver, which will result in the formation of more AMP and lower utilization of citrate for lipogenesis leading to higher energy state in the cell, i.e. higher concentration of ATP is more favourable for F-1,6-bisPase activation. However, the effect of fructose-2,6-bisphosphate is not very clear at present. The effect of the antidiabetic compounds like vanadate, *Trigonella* seed powder and insulin in reversing the increased activity could be by the reversal of the above discussed metabolites towards normal values and increase in pathways providing and utilizing the substrates and regulation together with normalization of the redox state of the cell.

Glyoxalase I in liver and kidney

The changes in the activities of glyoxalase I from liver and kidney of diabetic animals are shown in Table 9 together with the effect of antidiabetic compounds. In liver, the glyoxalase I activity decreased significantly and was restored to almost control levels by treatment with antidiabetic compounds (unpublished observations). In kidney, the glyoxalase I activity increased. This pattern agrees with the results published earlier (48) confirming that the glyoxalase enzymes are increased significantly in the diabetic tissues such as lens, kidney and RBS. A combined treatment of vanadate and *Trigonella* seed powder to diabetic animals showed the best reversal patterns.

The present and earlier data clearly indicate that trace element therapy, either alone or in combination and treatment with plant extracts may prove effective in the treatment of metabolic disturbances of the diabetic state. However the mechanism of action of these trace metals like lithium and vanadium for their insulin like effects and administration of various plant extracts remain unclear at present.

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